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Review

Genomics in cardiac metabolism

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Cell biology is in transition from reductionism to a more integrated science. Large-scale analysis of genome structure, gene expression, and metabolites are new technologies available for studying cardiac metabolism in diseases known to modify cardiac function. These technologies have several limitations and this review aims both to assess and take a critical look at some important results obtained in genomics restricted to molecular genetics, transcriptomics and metabolomics of cardiac metabolism in pathophysiological processes known to alter myocardial function. Therefore, our goal was to delineate new signalling pathways and new areas of research from the vast amount of data already published on genomics as applied to cardiac metabolism in diseases such as coronary heart disease, heart failure, and ischaemic reperfusion.

1. Introduction

This review aims both to assess and critically review the main results obtained in genomics of cardiac metabolism by the end of 2007. Although genomics have been differentially defined, we use the most widely accepted one, which was utilized by Gibson and Muse.[1] Genomics covers the overall structure or expression of our genetic inheritance including molecular genetics, transcriptomics, proteomics, and metabolomics.

The present work aims to delineate new signalling pathways and new areas of research from the vast amount of data already published on genomics as applied to cardiac metabolism in diseases, such as coronary heart disease (CHD) and heart failure (HF). This review was clearly limited by the fact that both the new molecular genetics, based on genome-wide association studies (GWAS) and metabolomics are still in their infancy, at least in the cardiovascular (CV) field. In contrast, the science of transcriptomics in the CV arena is more mature and had been developed in more detail.

From the growing flow of new data, a selection was made to illustrate better the potential of such a global approach. (i) Genetics has presently reached a new era based on GWAS. GWAS is, in principle, more liable to identify low-effect genes operative in pathological pathways and disease susceptibility in common diseases.[2] (ii) Transcriptomics is the study of gene expression of either transcripts or proteins. Gene expression is a short-term approach and is based on two main techniques, namely microarrays analysis and proteomics.[1] Two different aspects of transcriptomics have been developed herein: (a) modifications observed during the time course of a chronic disease of the heart and (b) pre- and post-conditioning and changes induced by short-term metabolic interventions (such as anaesthesia) that confer cardioprotection. (iii) The final chapter is on metabolomics that aims to quantify still more rapid modifications in metabolic compounds, but again on a genome-wide scale with the potential of medical applications.

It is worthy to note that with regards to the importance of the research area, we have selected studies, which were the most informative in the field of cardiology.

It has to be underscored that the majority of the studies reported herein, imply a technical approach, which is both costly and frequently requires large groups of investigators, patient cohorts (for GWAS) and multidisciplinary approaches (for metabolomics). Briefly, GWAS is principally based on high-density genotyping arrays that combine the power of association studies with the systematic nature of genome-wide research. Transcriptomics is mainly based on microarray analysis, i.e. a high-throughput method for screening a collection of microscopic DNA spots attached to a solid surface and to measure the expression levels of large numbers of genes in different samples simultaneously. Proteomics commonly utilized two-dimensional polyacrylamide gel electrophoresis to separate proteins, but there are also

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many other technical approaches available including protein microarrays. Finally, metabolomics is mainly a multidisciplinary approach based on the combination of pyrolysis and different spectrometry.

2. Are genome-wide association studies ready for common diseases?

Medical genetics not only involves the identification of one specific gene associated with a severe risk of having a given disease, but also provides information on associations of gene variants, each providing a moderate risk.[3–6] GWAS is based on (i) the availability of dense genotyping chips made with single-nucleotide polymorphisms (SNPs) (100 000–500 000, and recently, one million) covering most of the genome unequally; (ii) the growing resources of the International HapMap consortium (2007) which documents linkage disequilibrium (LD—a non-random association between alleles in a population due to their tendency to be co-inherited because of reduced recombination between them. Haplotype is the combination of alleles at neighbouring SNPs. Haplotypes blocks are the apparent haplotype structures of recombining portions of the genome in which blocks of consecutive co-inherited alleles are separated by short boundary regions), and is a public resource of common SNPs capturing most of the common genome sequence variability. In the human genome, there are 3.2 billion base pairs and approximately 15 million SNPs, indicating that kits using 500 000 SNPs should cover $<0.2\%$ of the genome.[4] The second generation human haplotype map now covers over 3.1 million SNPs.[7] Nevertheless, the statistical association among groups of SNPs, i.e. haplotype blocks, suggests that the identification of a few of the SNPs within the blocks can unambiguously identify all associated SNPs without the need to measure them directly. Recent studies have shown that the human genome is organized into a succession of ancestrally conserved distinct haplotype blocks. On the basis of this assumption, it is assumed that a 500 000 SNP scan should cover approximately 90% of the genome.[8] This chapter on genetics has been deliberately limited to GWAS and aims both to take a critical look at the main results obtained by the end of 2007 in CV research and to generate a new working hypothesis.

2.1 Main results, new insights into metabolic genomics

GWAS is, for the moment, dominated by the results of big groups such as The Wellcome Trust consortium (WTCCC)[9] and the Framingham Heart Study (FHS).[10] The WTCCC focuses on seven common diseases and includes CHD, diabetes, and hypertension. Most of these results were duplicated, especially those concerning CHD in a collaborative study with the German MI Family Study.[10,11] FHS comprises several working groups each describing specific associations with various traits [biomarkers, body mass index (BMI), and so on]. Several of these results were also duplicated. The WTCCC and FHS utilized approximately 500 000 and 100 000 SNPs, respectively. In addition, there are others groups that usually used smaller SNP density than the WTCCC.

To summarize (*Table 1*), GWAS has generated, for the time being, three groups of results.

(i) Diseases with phenotypic variance mainly due to genetic factors such as type 1 diabetes. GWAS documented at least a dozen genes strongly associated with type 1 diabetes (*HLA* class genes, *insulin* gene, *CTLA4* locus, *PTPN22*, and the *IFIH1* region) that mostly belong to the immune system which are the most important targets for research on type 1 diabetes.

(ii) On the other hand, diseases such as arterial hypertension and hyperlipidaemias remained poorly associated to simple genetic factors. Despite a considerable hope, genetic influence is weak and there are few risk alleles of large size effects and GWAS has been unable to identify with certainty susceptibility genes of modest effect size and we need, in the least, genotypic resources of increased density.[12] Long-term averages of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and blood pressure are highly heritable. Nevertheless, there are no significant associations that could help further research.

(iii) Type 2 diabetes, obesity, and atherosclerosis are in an intermediary position. GWAS has succeeded in detecting new loci of interest, which were strongly and reproducibly linked to the phenotype. Several new loci and genes of interest have now been identified:

  (a) The association of the 9p21 locus with CHD has been found by every GWAS published so far which suggests, at least, that this association is widely distributed. The locus contains two cyclin-dependent kinase inhibitors, which regulate cell cycles. Interestingly, the cell cycle pathway, including cyclins, was also over-represented in a large-scale gene expression study, which analysed pathways involved in atherogenesis using a modular approach. For these authors, their data suggest that smooth muscle de-differentiation is a key determinant in atherogenesis, which is new and unexpected.[13]
  (b) FHS has uncovered unexpected genetic associations with various markers of arterial stiffness, including large arteries calcium content and the reflected wave with candidate genes such as *LOXL2*, an oxidase involved in collagen cross-linking and arterial elasticity.
  (c) More than 100 publications have reported associations between genetic variants and BMI, and/or type 2 diabetes, but few have been reproduced so far. The two phenotypes are highly multigenic. Several associated genes such as *TCF7L2, KCNJ11, SLC30A8, EXT1* have been linked to pancreatic development and function.

2.2 Critical evaluation of genome-wide association studies

GWASs are still in their infancy and many technical and statistical problems remain to be solved. The results of promising studies such as FHS are still fragmented and require replication. Nevertheless, GWAS is probably the best tool now available to validate candidate gene associations and to enable unbiased searches for novel variants.

There are approximately seven million variants at a frequency $>5\%$, hence rare alleles may be overlooked.

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Table 1 Genome-wide analyses in diseases known to modify cardiac function directly or indirectly

Chromosome	Genes present in the locus	References
Myocardial infarction		
9p21.3	*CDKN2A* and *B* (cyclins of the cell cycle)	9–11,69,70
6q25.1	*MTHFDH* (methionine metabolism)	9,11
1p13.3; 1q41; 10q11.21; 15q22.33	No genes of interest	9,11
Subclinical markers of atherosclerosis		
	Associations found Carotid thickness with *AB12*, *PCSK2*, and *NOS3* Aortic calcium with *LRRC18* Ankle brachial index with *PFTK1*	10
Type 1 Diabetes		
2q24	*IFIH1/MDA5*	71,72
2q33	*CTLA-4*	9
1p13	*PTPN22*[a]	9,71,73
4q27	*IL-2* and *21*	9,73
6p21	*Major histocompatibility complex (MHC) (HLA-DRB1)*	9,73
10p15	*CD25*	9
11p15	*INS* (insulin)	73
12q13	More than 10 genes with presumed roles in immune signalling: *SH2B3* (P 10^{-12}); *ERBB3; SH2B*	9,71
12q24	*adaptor protein-3; TRAFD1; PTPN11*[a]*; CD69; CLEC; R262W*	9,71
16p13	*KIAA0350*[b] block, flanked by *CIITA* and *SOCS1*	9,71,73
16p13	*PTPN2*[a]	9,71
18p11	*PTPN2*[a]	9,71
18q22	*PTPN2*[a]; *CD226*	71
Type 2 Diabetes		
3p25	*PPARG, P12A*	9
11p15	*PPARG, P12A*	10
10q25	*TCF7L2*	9,10,74
16q	*FTO*	9,75
6p22	*CDKAL1*	
Clusters on Chromosome 10	*HHEX*; *IDE*	9,74
8	*SLC30A8*, *EXT2*; *LOC387761* (genes involved in pancreas development)	9,74
9p21.3	*CDKN2A* and *B*	9
	Associations with diabetic traits in 416 SNPs; verify replicated associations with *ABCC8*	10
Obesity and body mass index – Heritability around 60%		
	Associations with BMI and waist circumference in two SNPs; several candidate genes: *ESRI, PPARG, ADIPOQ, INSIG2, LEP, ESRI, SSTR2* and also *LRP1B, VIP, ADRB1, NPY2R, HSD3B1, ADRA1B, IL6R, AGTR1, FSHR*	10
16	*FTO*	18e
Arterial hypertension		
None		9
	Associations with blood pressure or arterial stiffness in seven SNPs; a few candidate genes: *MEF2C, SYNE1, LOXL2, TNFSF11*	10
Hyperlipidaemia		
	Associations with LDL-C, HDL-C, and triglycerides in seven SNPs; no new locus identifiable	10

Gene nomenclature can be found on 'genecards.org'. The papers from FHS are quoted in the work of Cupples *et al.*[10]

[a]The *PTPN* family plays a major role in insulin, immune signalling, and autoimmune diseases. *PTPN* can indirectly dephosphorylate *STAT1*, a major regulator of immune signalling.

[b]*KIAA0350* is a widely expressed gene of unknown function. Exon 12 may encode an immune receptor—ITAM—that binds the SH2B3 lymphocyte adaptor protein.

Good examples may be found in both the WTCCC (for *APOE* and *INS*) and FHS (for *GCKR*).[9,10] GWAS is based on both SNPs density and haplotype map, and can be improved by increasing SNP scan density or improving the haplotype map. The second-generation human haplotype map now covers over 3.1 million SNPs instead of one, and would provide the solution (see http://www.hapmap.org).

The second limitation lies in the fact that GWAS has many caveats when studies were conducted in less severe and more multifactorial diseases, and, in fact, the real problem is probably that GWAS did not catch RNA genes or regulatory segments (see Conclusions) that have not already been identified and could be major determinants in traits generation.

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3. Metabolic gene expression in cardiac hypertrophy and failure

Most of the work, which has been published so far in this area has been mainly descriptive and because they are dealing with the whole genome expression, only a few of them have clearly isolated the metabolic family of genes from the others. However, we now collect these data and attempt to draw some conclusions.

3.1 Transcriptomics

Genomic approaches such as transcriptional profiling by DNA microarrays allow the simultaneous analysis of some 55 000 transcripts in a single assay[14–16] and provides both qualitative (switched on/off genes) and quantitative data (transcriptional level of single genes), so that subtle differences on gene activation can be detected. Today, transcriptional analysis can be performed on minute tissue samples and, one of the limits due to cellular heterogeneity can now be resolved by laser microdissection, which allowed studies of one cell population. Nevertheless, profiling whole CV tissue samples may generate novel hypotheses, and help to identify unexpected cell components and reveal novel marker genes. As reviewed by Nanni *et al.*,[17] a growing number of transcriptome microarray studies have been applied to CV diseases with the aim of recognition of specific disease phenotypes to improve both prognosis and therapeutic assessment.

The comparative study performed by Gao *et al.*[18] between canine tachy-pacing, mouse transgenic, and human HF reveals that, in humans, the disease involves a downregulation of genes in a broad range of biological processes. In contrast to this, in experimental models of HF, downregulation of energy metabolic pathways is observed. Human ischaemic HF and canine HF share a similar over-representation of transcriptional pathways in the upregulated genes. However, in this study, no induction of prominent HF markers, e.g. atrial natriuretic peptides (ANP) and brain natriuretic peptides (BNP) was detected.[19] In the rat heart following coronary ligation, Laframboise *et al.*[20] demonstrated that transcripts for signal transduction and inflammation gene expression dominated in the infarct zone during late-term recovery. There was recruitment of genes for transcription, metabolism, and detoxification—all classes were depressed in the day 1 infarct zone. In contrast, within one day, the remote zone exhibited an upregulation in many genes particularly in those of the metabolism family or those associated with developmental processes. In contrast, transcripts for contractile proteins matched control values. In the late-term, the metabolic responses of the remote zone was attenuated.

In humans, Kittleson *et al.*,[16] developed a strategy to identify genes differentially expressed between ischaemic (ICM) and non-ischaemic (NICM) cardiomyopathy. When compared with controls, 257 genes (over the 22 000 transcripts present on the Affymetrix microarray platform) were differentially expressed in NICM and 72 genes in ICM. Only 41 genes were shared between NICM and ICM, and they were mainly involved in cell growth and signal transduction (*Table 2*). Those specifically expressed in NICM were frequently involved in metabolism and included *ACE2* and genes involved in fatty acid (FA) and cholesterol metabolism. The genes specifically upregulated in ICM more often had catalytic activity, such as *SERPINB1*, *SERPINE1*, *ATP1B3*. Besides these results, using a CardioShip (CardioChip is a custom-made CV-based tag glass slide cDNA microarray formed by non-redundant 10 848-element human CV-based expressed sequence) in human HF, Barrans *et al.*[21] found more than 100 transcripts upregulated, including stress-response proteins, transcription/translation regulators together with the classical HF genes like ANP, and selected sarcomeric and extracellular matrix (ECM) proteins. Conversely, they found a downregulation of cell-signalling channels and mediators, particularly those involved in the Ca^{2+} pathways of crucial importance. In patients with moderate HF and dilated cardiomyopathy (DCM), the transcriptional profiles demonstrated a switch in the cardiomyocyte energy pathways (higher rate of lipid oxidation), apoptosis, and a downregulation of cell cycle-controlling genes.[22] In addition, alterations in the intracellular signalling functions were already present in the early stages of the disease. The genes regulating muscle contraction were deregulated in intermediate stages, whereas apoptosis and the cell cycle regulator gene expression were altered in the late stages.

Different results were obtained by Sanoudou *et al.*[23] The genes of energy metabolism were predominantly underexpressed in DCM and hypertrophic cardiomyopathy (HCM), but overexpressed in ICM.[23] For instance, activation of the cardiac Peroxisome Proliferator-Activated Receptor-alpha (PPAR-alpha) by Peroxisome Proliferator-Activated Receptor-gamma Coactivator 1-alpha (PGC1-alpha) induces the expression of genes encoding for proteins involved in FA uptake, transport into mitochondria, and beta-oxidation.[24] The increased FA utilization leads reciprocally to a decrease in glucose metabolism. On the other hand, the well-known hypoxia-inducible transcription factor 1-alpha (HIF1-alpha) targets approximately 70 genes and among them those that increase oxygen delivery and survival during hypoxia (such as genes involved in the upregulation of glucose metabolism).[25] HIF1 alpha is one of the few transcription factors promoting upregulation of the glucose pathway. The downregulation of PPAR-alpha and PGC1-alpha in hypertrophy and in HF could also be considered beneficial, because it indirectly

Table 2 Distribution of genes significantly altered in human cardiac disease

Family genes	Up	Down
Nucleus	1	
Metabolism	4	1
Cytoskeleton	1	1
Cell adhesion, extracellular matrix	1	1
Inflammatory and immune response	2	None
Binding	3	None
Signal transduction	5	None
Cell growth	4	None
Development	2	None
Catalytic activity	1	None
Apoptosis	1	None

Significant changes in gene expression specific to either ischaemic or non-ischaemic cardiomyopathies matching sham cohort verified in different independent studies (reviewed in Kittleson *et al.*[16]). The columns indicated the activated (up) and inhibited (down) genes comprising each individual gene classification.

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favours glucose utilization instead of FA oxidation. Finally, the increased cytoplasmic Ca^{2+} during diastole in most forms of end-stage HF permeates the mitochondrial matrix, where it stimulates Ca^{2+}-regulated key enzymes in the tricarboxylic acid cycle as well as ATP synthase, thus accelerating the energy-producing metabolism.[26]

In vivo measurements of high-energy phosphate compounds have shown that the failing heart is an engine out of fuel.[24,27] No consent exists, however, whether the FAs or the glucose pathway predominates in end-stage HF. This may, in part, be due to the different pathophysiological aetiologies leading to a final common HF syndrome exhibiting more generalized metabolic dysfunction, rather than alterations of specific substrate preference regulating genes.[24]

Transcriptomic analysis assesses that HF, independent of its aetiology, is characterized by some common final patterns of gene expression, including those coding for a high rate of lipid oxidation with low glycolysis, a dysregulated apoptosis, cell-cycle regulator genes, and ECM remodelling. It was suggested that different gene expression patterns were associated with clinical HF severity[14] and some patterns characteristic of clinical syndromes[16] may open the road for aetiology-specific therapies in NICM-targeting metabolic pathways. In most of these genome-wide analyses, no firm correlation has been established between the altered expression of specific genes with functional parameters.

3.2 Transcriptomics and signalling

Most of the extracellular stimuli (ions, hormones, cell mediators, and mechanical signal) are integrated and transmitted by various intracellular signalling pathways to the cell nucleus, ultimately affecting gene-expression patterns.[26,28] The signalling pattern in response to stimuli may represent an early and sensitive disease-specific fingerprint before the final cellular phenotype has fully developed.

A large body of literature based on *ex vivo* and *in vivo* animal models indicates that pathological myocardial remodelling is mainly induced by neurohormonal factors (including angiotensin-II, endothelin-1, and catecholamines) through Gq-coupled (G-protein-coupled receptors, GPCRs) signalling pathways.[28,29] Downstream of Gq, the pathway involves phospholipase-C beta, which hydrolyses phosphatidylinositol bisphosphate (PIP2) into diacylglycerol (DAG) and inositol trisphosphate (IP3). IP3 releases calcium from intracellular stores, which may activate the phosphatase calcineurin-NFAT pathway. In concert, DAG activates protein kinase C (PKC) family members, some of which contribute to hypertrophic gene expression.[30] In contrast, exercise-induced hypertrophy appears to be regulated through the PI3K (phosphoinositide-3 kinase)—PKB/Akt—GSK3alpha/beta (glycogen synthase kinases) pathway. Growth (GH) hormone and insulin-like growth factor (IGF) are the major stimuli for physiological hypertrophy.[31–33] Of note, these signalling pathways are complemented by a panoply of interconnected routes including the mitogen-activated protein kinase (MAPK) cascades and the Janus kinase/signal transducer and activator of transcription JAK/Stat pathway.[26,34] Additional signalling components also contribute to the final phenotype.

Genome-wide transcriptomic analysis of differentially regulated genes in physiological (dubbed 'adaptive') and pathological (or 'maladaptive') hypertrophy, as well as in HF of rats was recently reported.[35,36] Taken together, the gene activity profiles obtained with Affymetrix Rat Genome U34A microarrays pointed out that (i) gene clusters typically changing in adaptive hypertrophy predominantly comprised genes involved in metabolism and cell growth; (ii) maladaptive hypertrophy was characterized by changes in gene clusters associated with oxidative stress responses, inflammation, and apoptosis; (iii) transition to overt HF was accompanied by an increase in those genes already affected in compensated pathological hypertrophy, by recruitment of additional signalling genes, such as *GATA4*, *RAB7*, *NRAS*, *GNA12*, *STAT3*, *STAT5B*, *FYN*, *CRKO*, *MYCN*, *PTEN*, *AKT1*, and *IL6ST/gp130*.

The most striking and potentially physiologically meaningful observation concerns the shift in metabolic gene expression.[35] Several genes involved in beta-oxidation of lipids are upregulated in adaptive, but downregulated in maladaptive hypertrophy. On the other hand, a number of genes stimulating glucose metabolism are selectively upregulated in adaptive but not in maladaptive hypertrophy. Furthermore, the uncoupling protein UCP2 is downregulated in adaptive and upregulated in maladaptive hypertrophy implying that ATP production through oxidative phosphorylation might be more effective in exercise-trained hearts. Collectively, these findings support the notion that transcriptomic changes comprise the basis for functional improvement of cardiac capacity in glucose utilization by adaptation to exercise. It remains to be established whether some or all of the expression changes typical for pathological cardiac hypertrophy can be observed and therapeutically addressed in humans. One major obstacle presents the much slower development in most clinical heart diseases coupled with small alteration in the early phases, which might escape detection.

3.3 Proteomics

There are approximately 10 times more proteins than genes, and expression arrays should preferably be complemented by proteomic evaluation because the transcripts are not always reflected in corresponding protein accumulation, and also because protein may undergo variable co- and post-translational modifications, the information for which is not contained in the original transcript. Therefore, new findings discovered by transcript profiling, may serve as leads but require subsequent functional characterization.[23] For both the microarray analysis and proteomics, an unbiased approach requires sophisticated bioinformatics tools and a large panel of controls which will not be detailed in this review (see details in Gibson and Muse,[1] Ruiz and Witt,[19] da Silva *et al.*,[37] and Lucchinetti *et al.*[38]).

Proteomic biomarkers differ from traditional biochemical markers, in which multiple interacting protein species are evaluated simultaneously to reflect the response of a cell or an organism to disease. Proteomics might then be utilized to investigate rapid changes in signalling pathways. A resource of web-based two-dimensional electrophoretic maps annotated for healthy and diseased cardiac tissues has recently been highlighted.[39] Proteomics includes other approaches such as gel-free separation (LC, liquid IEF, CE, FFE) and identification of the purified protein can be performed through mass spectrometry (MS or MS/MS).

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Lindsey *et al.*,[40] using a multi-dimensional proteomic approach, identified 123 proteins that were differentially expressed during left ventricular hypertrophy in mouse, including LIM proteins, thioredoxin, myoglobin, FA-binding protein 3 (FABP), and myofibrillar proteins. The classification into seven categories is as follows: (1) cell structure and motility; (2) cell signalling and communication; (3) metabolism; (4) transcription, translation, and trafficking; (5) cell division; (6) cell and organism defense; (7) unknown provides more information. Among the 95% that could be assigned to one of the six known functions, 36% and 21% of the identified proteins belongs to metabolism, cell structure, and motility classes, respectively. Some of these changes were not confirmed using western blotting, as changes in myoglobin. Besides, through a subproteomic analysis, Banfi *et al.*[41] demonstrated, among the metabolic changes observed in human HF, a decreased FA oxidation as indicated by the FABP content.

3.4 Critical evaluation of transcriptomics and proteomics

Both transcriptomics and proteomics are no more in their infancy and require many technical and statistical problems to be solved. As already pointed out above, the results are still fragmented and require further development. One might pay attention to the cell heterogeneity of the heart, and consider the part of non-muscle cell (endothelial cell, fibroblast) into cell signalling changes. Nevertheless, proteomics emerges as one of the best tool to bring new insight into applied genomics of cardiac metabolism, mainly through the analysis of post-translational modifications. Yet, proteomics remains to be a field strongly based on technologies.

4. Metabolomics

The study of the collection of small molecular-weight organic and inorganic species present in a biological system is defined as metabolomics.[42,43] These collection of metabolites can provide a phenotypic oversight of the organism, either as a snapshot in time or as an integrated picture of biology over a period of time. The human metabolome is estimated to contain approximately 1500 endogenous metabolites not including many lipids and gut microflora-derived metabolites,[44] and is chemically and physically diverse.[45] Metabolites are the final downstream product of gene transcription and so reflect more closely the cell activity (or phenotype) at a functional level. Furthermore, metabolic control analysis (MCA) describes that small changes in enzyme activity (and the transcripts which encode these) may have minimal influence on metabolic fluxes, but large influences on the concentrations of metabolites. Consequently, the metabolome is thought to be a potentially more sensitive marker of cellular processes both in normal physiology and disease.[42]

The science of metabolomics operates to a workflow or pipeline approach[46] and involves multi-disciplinary teams with the objective to create valid and experimentally robust data and convert this data into biological knowledge.[47] Analogous to the human genome project, there are numerous approaches to define the human metabolome[44] (http://www.husermet.org; http://www.hmdb.ca).

4.1 Biomarker detection

The application of metabolomics to human disease studies is an emerging science, in which patterns of metabolites in the disease state are compared with those of 'healthy' individuals to allow identification of potential biomarkers of the disease process.[48,49] An advantage of using metabolomics for the identification of biomarkers of a disease state are the inductive approaches applied to studying these complex systems, where many diseases are characterized as multiple disorders or display multiple phenotypes across a population.

In a recent study from our group, the serum of 52 patients with documented HF (left ventricular ejection fraction $<40\%$) was subjected to metabolomic analyses. Multiple biomarkers were detected, of which 2-oxoglutarate and pseudouridine were the metabolites showing greatest statistical differences between the case and matched control classes. The combination of both provided a greater sensitivity and specificity for the diagnosis of HF in this patient cohort than the current gold standard biomarker BNP.[50] Pseudouridine is a modified nucleoside that is found in ribosomal and transfer RNA, is produced post-transcriptionally and is considered to be an excellent measure for RNA degradation, and hence the cell turnover. Tumour cells exhibit an unusually high turnover, and consequently has also been proposed as a tumour marker[51] where it can have significant prognostic value. In HF its raised level may, in part, reflect the remodelling process in the heart itself or increased catabolic activity in peripheral tissues.

2-Oxoglutarate is an important intermediate of the Krebs cycle and one of the 12 major precursors for the synthesis of most biochemical substances. In recent years, it has become increasingly clear that alterations in energy metabolism may contribute to the pathophysiology of HF.[52,53] The raised levels of 2-oxoglutarate seen in HF may reflect a decreased flux through the Krebs cycle in HF and overflow of some metabolites into the circulation.

In another study in which blood samples were analysed from subjects undergoing exercise stress testing, a number of biomarkers were identified in those patients whose stress test demonstrated evidence of cardiac ischaemia[54] and noted that six of the metabolites in the Krebs cycle were significantly over-represented in the 23 markers of cardiac ischaemia identified. Indeed, in experimental animal models of cardiac ischaemia it has been demonstrated that there is a significant reduction (80%) in efflux of Krebs cycle intermediates from the myocardium with maintenance of intracardiac tissue levels and it is thought that this may be an important pathophysiolocal mechanism to preserve Krebs cycle metabolic intermediates and therefore protect ATP production within the myocardium.

Metabolomic analyses in CV medicine may not only be potentially useful in the identification of potential biomarkers of CV disease in the future, but may provide information regarding prognosis, response to therapy, and underlying mechanisms of the disease process. It is worthy to note that to reach this goal, the relative specificity for CV diseases of the current metabolomics approaches need to be improved.

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5. Genomics and proteomics applied to myocardial ischaemia and cardioprotection

5.1 Genomics applied to cardioprotection

5.1.1 Cardioprotection obtained by ether-derived halogenated volatile anaesthetics

Ischaemia, and more recently anaesthesia, are known to confer cardioprotection on either a short- or a long-term basis through different mechanisms. The well documented pre- and post-conditionings (Pre-C and Post-C) are good examples of how genomics can be applied to CV research. The induction of short- and long-term gene reprogramming was examined by genome-wide gene activity profiling after either ischaemia or anaesthesia obtained with ether-derived halogenated volatile anaesthetics (VAs).[37,55] Alterations in gene expression induced by anaesthetic conditioning were compared with the well-known ischaemic conditioning procedures (*Figure 1*). Subsequently, the insight gained from the experimental approaches could be directly translated into clinical applications. Thereby, the volatile cardioprotective sevoflurane in comparison with the widely used intravenous propofol, reduced the expression of genes involved in noxious pathways and shifted the energy metabolism away from FA oxidation more to glucose utilization.[38]

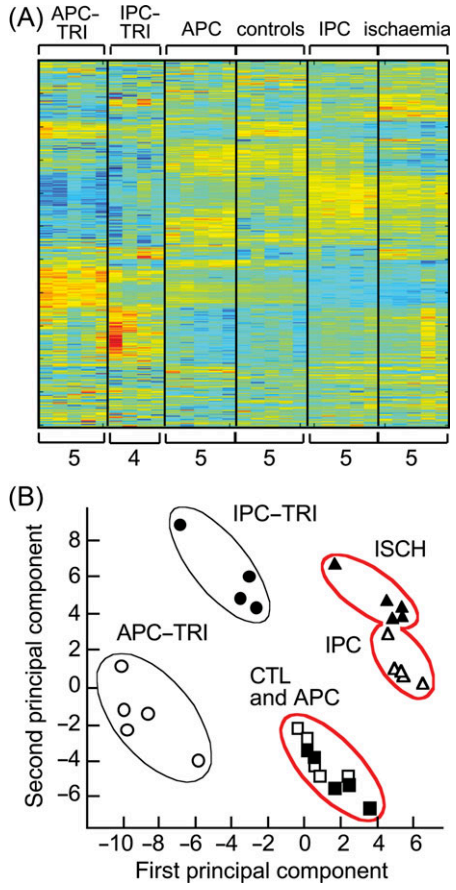

Figure 1 Transcriptome profiles of isolated beating rat hearts after conditioning trigger alone and after test ischaemia. (*A*) Global gene expression matrix (heat map) of 2212 ANOVA-filtered genes (with *P*-value = 0.01). Horizontal rows correspond to genes and vertical columns correspond to samples (numbers of chips on the abscissa of heat map) in the six treatment groups. Colours (dark blue indicates least and red indicates highest degree of expression) represent quantification for gene expression based on normalized and centered robust multichip average values. Coupled two-way clustering for all the six treatment groups (not shown) yielded eight stable gene clusters. (*B*) Principal component analysis of the six treatment groups representing two-dimensional vector projection of individual chip data. APC-TRI, application of 15 min isoflurane (in clinically relevant concentrations) without following test ischaemia (anaesthetic trigger); IPC-TRI, application of three cycles of 5 min ischaemia interspersed by 5 min reperfusion periods without following test ischaemia (ischaemic preconditioning trigger); APC, anaesthetic preconditioning followed by 40 min of global ischaemia; controls (CTL), time-matched perfusion without any treatment; IPC, ischaemic preconditioning followed by 40 min of global ischaemia; ischaemia (ISCH), 40 min global ischaemia without preconditioning. Tissue samples for chip analysis were taken after 3 h of additional perfusion time subsequent to the treatments. Treatment groups are encircled. Note: overlapping and nearby located treatment groups are encircled in red. Modified from da Silva *et al.*[37]

Important differences were observed in signalling between ischaemia and VAs-induced conditioning.[37,56] The two major signalling routes for VAs-Pre-C were the phospholipase-C pathways and a direct activation of nitric oxide synthase. Both signalling routes converge on activating the mitoKATP channels and blockade of these channels by 5-hydroxydecanoate results in complete inhibition of cardioprotection. Conversely, application of the specific channel opener diazoxide induces cardioprotection just like the VAs.

In contrast, signalling by the ischaemic Pre-C trigger also involves the MAPK pathways in addition to those activated by VAs.[37,57] Differences in signalling between the two modes of Pre-C were expected, because the ischaemic trigger exerts a noxious cellular stress for induction of protective responses, while the VAs trigger rather sedates the cells without causing any lesions,[58] and as a corollary, the two trigger mechanisms affect the gene activity profile in different ways (*Figure 1*).[37,59]

The major signalling routes by anaesthetic and ischaemic Post-C comprise the so-called reperfusion injury salvage kinase pathways (RISK).[60–63] The RISK pathways comprise several signalling routes all of which converge on inhibiting the mitochondrial permeability transition pore (mPTP) by an as yet unknown mechanism. Therefore, the functionally coupled mitoKATP channel and mPTP represent the major mitochondrial target structures for pre- and post-conditioning, respectively.[58,64] Finally, functional impairment of either or both structures can initiate apoptosis/necrosis.

5.1.2 Transcriptomic profile during VAs-induced cardioprotection

From visual inspection of the heat map (*Figure 1A*, first two column groups), one can conclude that both triggers regulate a number of genes in parallel (same colour in horizontal rows), while a large portion of genes were differentially regulated as indicated by non-corresponding colour rows. On the other hand, a large portion of genes display a similar pattern after test ischaemia alone and after ischaemic Pre-C followed by test ischaemia (*Figure 1A*, last two column groups). Finally, a similar correspondence of commonly regulated genes appears in time-matched perfusion controls when compared with VA Pre-C followed by test ischaemia (*Figure 1A*, middle two column groups). The different activity profiles between the two conditioning triggers and the substantial overlapping of the profiles between (i) VAs Pre-C and untreated controls and (ii) ischaemic Pre-C and unprotected ischaemia are visualized in *Figure 1B*.[37] These findings indicate different gene-activity profiles for the two trigger mechanisms. Although ischaemic Pre-C is able to afford cardioprotection, the subsequent ischaemic cell damage incurred by the test ischaemia brings the

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profiles surprisingly close to those of unprotected hearts subjected to severe ischaemia. One may speculate that a number of the cell reactions induced by ischaemic Pre-C are similar or identical to those induced by severe ischaemia in unprotected hearts, thus contributing to the similar transcriptome profiles. This constitutes a compelling reason to not apply ischaemic Pre-C therapeutically in the clinical setting for patients with an already compromised vascular system. On the other hand, the completely overlapping profiles of VAs Pre-C with those of untreated perfused control hearts (*Figure 1B*) indicates that this procedure does not afflict cell damage in order to induce cardioprotection and may well have clinical potential in perioperative medicine.

5.2 Proteomics and ischaemia reperfusion

Proteomic investigations have been used to reveal alterations in mitochondrial signalling mechanisms in different cardiac phenotypes.[65] Proteomic analysis of ischaemic/reperfused rabbit hearts revealed multiple changes associated with stress responses and energy metabolism in mitochondria. Interestingly, the mitochondrial subproteomic alterations correlated with susceptibility to injury, suggesting that mitochondrial signalling might also serve as biomarker of CV impairments.[65] Proteomics studies of the mitochondria have provided novel evidences for kinase signalling cascades localized in the mitochondria, some of which are known to involve various isoforms of PKC.[66] Finally, post-translational modifications of proteins, protein–protein interactions and the identity, localization and function of signalling complexes can be monitored by proteomic techniques. Agnetti *et al*.[66] provided new concepts related to novel cardiac post-translational modifications including (i) PKC-mediated phosphorylation of a myofilament component, troponin I, and of intermediate filaments, desmin; (ii) novel protein modifications that are related to maladaptive cellular processes.

6. Limitations and perspectives

Large-scale quantitative analysis of gene expression, genome structure, and metabolic by-products are now applied to the CV field. Genomics is now producing billions of new data and there is an urgent need for classification, ordering, and finally functional interpretation. At this moment, we are facing two types of problems: (i) how to sort such a flood of new data; (ii) how to explain the fact that GWAS, despite its enormous potential, fails to find more readily disease-related variants of interest.

Traditional approaches have been based upon reductionism, whereby small parts of a larger complex system can be investigated by hypothesis-driven experiments. However, in systems of high complexity, a data-driven approach is now applied where an experiment is designed to collect data from which a hypothesis can be deduced.[43] Based on the various results coming from the different aspects of genomics, this inductive approach is applied extensively in the post-genomic era. One possibility, among others, to better link these new results to functional interpretation is modular biology. Biological systems are scale-free networks made from genes, proteins, or traits that interact with one another and form functional modules. Networks emerge according to the 'rich-gets-richer' mechanism with hubs and nexus as attractive candidate for targeting new pathways.[15,67] Such an approach has still been rarely applied.[13]

Presently, the changing definition of 'what is a gene' may contribute to the difficulty in capturing new variants of interest by the GWAS. To account for both the complex patterns of dispersed regulation and pervasive transcription uncovered by the ENCODE project and the abundance of non-coding regulatory RNA genes (as miRNA), it was proposed to define a gene as 'a union of genomic sequences encoding a coherent set of potentially overlapping functional products'.[68] Such a definition 'manifests how integral is the concept of biological function' and also highlights the limits of the new genetics. In that sense, we could predict that ENCODE, when fully available, will probably modify our approaches to genomic studies like how the two generations of the HapMap programme did.

7. Conclusions

Cell biology is in transition from a reductionistic approach to a more integrated science. Large-scale analysis of genome structure, gene expression, and metabolites are new technologies available for studying cardiac metabolism, including diseases known to modify cardiac function. These technologies have several limitations, which will hopefully be overcome in the near future. (i) GWAS are a revolution in molecular genetics and have allowed identification of new variants associated with cardiac metabolism in common CV diseases, including variants in the immune system in type 2 diabetes and markers of the cell cycle in atheroma. (ii) The transcriptome is modified in HF with a global change in gene families involved in signal transduction, cell growth, and metabolism with the shift in the metabolic genes being predominant irrespective of the disease aetiology. (iii) The VA-induced pre- and post-conditioning is a good example of how genomics can help to decipher the metabolic pathways involved in cardioprotection. (iv) Metabolomics is an emerging technique, which has already been able to identify biomarkers of interest, such as pseudouridine and 2-oxoglutarate in HF for example.

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Gene nomenclature can be found on http://www.genecards.org/

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